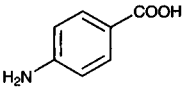
Aminobenzoic acid



Molecular formula: $C_7H_7NO_2$
Molecular weight: 137.14
CAS Registry No.: 150-13-0
Merck Index: 443

SAMPLE

Matrix: blood
Sample preparation: 100 μL Plasma or urine + 200 μL MeCN, vortex for a few s, centrifuge at 800 g for 5 min, inject a 5 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:0.04% pH 2.5 ± 0.05 phosphoric acid 3.5:96.5
Flow rate: 1.5
Injection volume: 5
Detector: UV 254

CHROMATOGRAM

Retention time: 8
Internal standard: p-aminobenzoic acid

OTHER SUBSTANCES

Extracted: iothalamate, p-aminohippuric acid

KEY WORDS

plasma; dog; human; p-aminobenzoic acid is IS

REFERENCE

Prueksaritanont,T.; Chen,M.L.; Chiou,W.L. Simple and micro high-performance liquid chromatographic method for simultaneous determination of p-aminohippuric acid and iothalamate in biological fluids, *J.Chromatogr.*, **1984**, *306*, 89–97.

SAMPLE

Matrix: blood
Sample preparation: 50 μL Serum + 50 μL MeCN, vortex for 15 s, centrifuge at 10000 g for 5 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 100 × 8 10 μm μBondapak C18
Mobile phase: 67 mM pH 2.8 phosphate buffer containing 5 mM tetrabutylammonium phosphate (low-UV Pic A Reagent, Waters) (Buffer was KH_2PO_4:Na_2HPO_4 97.5:2.5.)
Flow rate: 2
Injection volume: 20
Detector: UV 275

CHROMATOGRAM

Retention time: 13.0
Internal standard: p-aminobenzoic acid

OTHER SUBSTANCES

Extracted: p-aminohippuric acid

KEY WORDS
serum; p-aminobenzoic acid is IS

REFERENCE
Jenny,P.D.; Weber,A.; Smith,A.L. Quantitation of p-aminohippuric acid in biological fluids by high-performance liquid chromatography and dual-wavelength ultraviolet detection, *J.Chromatogr.*, **1989**, *490*, 213–218.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma + 150 μL salicylic acid in 1 M perchloric acid, centrifuge at 10000 g for 5 min, inject a 30 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.5 5 μm Cosmosil MS-C18
Mobile phase: MeCN:water:glacial acetic acid 3:100:1, adjusted to pH 4.0 with 10 M NaOH
Column temperature: 40
Flow rate: 1.5
Injection volume: 30
Detector: F ex 270 em 350

CHROMATOGRAM
Retention time: 8.32
Internal standard: salicylic acid (17)
Limit of detection: 50 ng/mL
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, p-acetamidobenzoic acid, p-acetamidohippuric acid, p-aminohippuric acid

KEY WORDS
plasma; rabbit; pharmacokinetics

REFERENCE
Song,D.J.; Hsu,K.Y. Determination of p-aminobenzoic acid and its metabolites in rabbit plasma by high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.B*, **1996**, *677*, 69–75.

SAMPLE
Matrix: cell cultures
Sample preparation: Condition a cyclohexyl-bonded silica Bond-elut SPE cartridge with 2 mL MeOH and 2 mL water. Centrifuge cell cultures at 6000 g at 4° for 15 min, add 100 μL supernatant and 100 μL 2 μg/mL sulfamerazine to the SPE cartridge, wash with 1 mL water, elute with 1.5 mL MeOH. Evaporate the eluate to dryness under a stream of air at 60°, reconstitute the residue in 100 μL water, vortex, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm ODS Hypersil
Mobile phase: MeOH:10 mM pH 2.5 phosphate buffer 5:95 containing 40 mM tetrabutylammonium bromide
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 4

Internal standard: sulfamerazine (7.5)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: sulfadiazine, trimethoprim, dibromopropamidine isethionate

KEY WORDS
SPE

REFERENCE
Taylor,R.B.; Richards,R.M.E.; Xing,D.K.-I. Determination of antibacterial agents in microbiological cultures by high-performance liquid chromatography, *Analyst*, **1990**, *115*, 797–799.

SAMPLE
Matrix: perfusate
Sample preparation: Adjust pH of 5-10 mL perfusate to 5 with 180 μL 2.5 M HCl, extract twice with an equal volume of ethyl acetate. Combine the organic layers, add 1 mL water, evaporate them to 1 mL under vacuum, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water containing 30 μL/L triethylamine, adjusted to pH 2.3 with phosphoric acid 10:90
Flow rate: 1.5
Injection volume: 20
Detector: UV 290

CHROMATOGRAM
Retention time: 3.8

OTHER SUBSTANCES
Extracted: procaine, aminohippuric acid, 4-acetamidobenzoic acid

KEY WORDS
rabbit; chinchilla; pharmacokinetics

REFERENCE
Henrikus,B.M.; Kampffmeyer,H.G. Ester hydrolysis and conjugation reactions in intact skin and skin homogenate, and by liver esterase of rabbits, *Xenobiotica*, **1992**, *22*, 1357–1366.

SAMPLE
Matrix: perfusate
Sample preparation: Prepare ultrafiltrate from 200 μL perfusate using an Ultrafree-MC unit with a 30000 MW cut-off (Millipore) with centrifuging at 2000 g for 20 min, inject a 50 μL aliquot.

HPLC VARIABLES
Column: Ultrasphere C18
Mobile phase: MeCN:water:acetic acid:triethylamine 12:88:1:0.05
Flow rate: 1.2
Injection volume: 200
Detector: UV 270

CHROMATOGRAM
Retention time: 2.85

OTHER SUBSTANCES
Extracted: N-acetyl p-aminobenzoic acid

REFERENCE
Derewlany,L.O.; Knie,B.; Koren,G. Human placental transfer and metabolism of p-aminobenzoic acid, *J.Pharmacol.Exp.Ther.*, **1994**, *269*, 761–765.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm TSK-gel ODS-80Ts (Tosoh Co., Japan)
Mobile phase: MeCN:water:acetic acid 10.5:89.5:1
Flow rate: 0.8
Injection volume: 20-30
Detector: UV 254

CHROMATOGRAM
Retention time: 11.7

OTHER SUBSTANCES
Simultaneous: carteolol

REFERENCE
Umehara,K.; Kudo,S.; Odomi,M. Involvement of CYP2D1 in the metabolism of carteolol by male rat liver microsomes, *Xenobiotica*, **1997**, *27*, 1121–1129.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 125 × 4 5 μm endcapped LichroCART RP18
Mobile phase: MeCN:7.5 mM pH 7.3 phosphate buffer 10:90
Column temperature: 40
Flow rate: 0.8
Injection volume: 30
Detector: E, ESA Coulochem II coulometric cell 5011, first electrode +450 mV, second electrode +630 mV; UV 254

CHROMATOGRAM
Retention time: 1.2

OTHER SUBSTANCES
Simultaneous: hydrochlorothiazide

REFERENCE
Richter,K.; Oertel,R.; Kirch,W. New sensitive method for the determination of hydrochlorothiazide in human serum by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.A*, **1996**, *729*, 293–296.

SAMPLE
Matrix: solutions
Sample preparation: Add 500 μL of a solution in MeCN to 100 mg finely powdered potassium carbonate, add 250 μL 3.8 mM 18-crown-6 in MeCN, add 250 μL 0.8 mM reagent in MeCN, heat at 80° in the dark for 20 min, cool, inject a 5 μL aliquot. (Synthesize the reagent, 3-bromomethyl-6,7-dimethoxy-1-methyl-2(1H)-quinoxalinone, as follows. Stir 483

g veratrole in 1.45 L acetic acid at 15° for 1 h, add 683 g concentrated nitric acid (d 1.05) over 1 h (maintain the temperature below 40° by cooling and regulating the rate of addition of the nitric acid). Continue stirring and add 2.127 L fuming nitric acid (d 1.50) over 1 h while maintaining the temperature below 30°, let stand for 2 h, pour into a large volume of cold water, filter, wash the solid with water until the washings are neutral, recrystallize from EtOH to give 4,5-dinitroveratrole (mp 129.5-130.5°) (J. Am. Chem. Soc. 1946, 68, 1536). Reflux 5 g 4,5-dinitroveratrole in 200 mL benzene (Caution! Benzene is a carcinogen!), add 100 g 60 mesh iron powder and 20 mL concentrated HCl in small portions over 1 h, reflux for 4 h, add 10 mL water, reflux for 2 h, cool, make alkaline with 2.5 M NaOH, extract several times with 200 mL portions of benzene. Combine the organic layers and evaporate them to dryness, add 10 mL concentrated HCl, recrystallize from EtOH to give 1,2-diamino-4,5-dimethoxybenzene monohydrochloride as very slightly pink needles (mp 240°) (Anal. Chim. Acta 1982, 134, 39). Heat 2.5 mmoles 1,2-diamino-4,5-dimethoxybenzene hydrochloride and 2.4 mmoles pyruvic acid in 30 mL 500 mM HCl on a boiling water bath for 2 h, cool with ice-water, filter. Wash the precipitate with water and dry it under vacuum, recrystallize from MeOH:water 90:10 to give 6,7-dimethoxy-3-methyl-2(1H)-quinoxalinone as yellow needles (mp 255°) (Chem. Pharm. Bull. 1985, 33, 3493). Treat 1 g 6,7-dimethoxy-3-methyl-2(1H)-quinoxalinone dissolved in 50 mL anhydrous MeOH with a solution of diazomethane in ether, evaporate to dryness under reduced pressure, dissolve the residue in 5 mL ethyl acetate, chromatograph on a 250 × 35 column filled with 130 g 70-230 mesh silica gel 60 (Merck) using n-hexane:ethyl acetate 25:75 to give 6,7-dimethoxy-1,3-dimethyl-2(1H)-quinoxalinone as yellow needles (mp 170-171°). Dissolve 350 mg 6,7-dimethoxy-1,3-dimethyl-2(1H)-quinoxalinone in 3 mL acetic acid, add 350 mg anhydrous sodium acetate, add 2 mL 1.5 M bromine in acetic acid, heat at 100° for 15 min, cool, add 10 mL ether, filter, wash the solid 2 or 3 times with small portions of ether. Combine the filtrate and washings and evaporate them to dryness, dissolve the residue in 5 mL ethyl acetate, chromatograph on a 250 × 35 column filled with 130 g 70-230 mesh silica gel 60 (Merck) using ether, evaporate the main fraction to dryness, recrystallize the residue from n-hexane:ethyl acetate 50:50 to give 3-bromomethyl-6,7-dimethoxy-1-methyl-2(1H)-quinoxalinone as yellow needles (mp 161-163°).)

HPLC VARIABLES

Column: 100 × 4 10 μm Radial-Pak C18 (Waters)
Mobile phase: Gradient. MeOH:water from 30:70 to 70:30 over 30 min
Flow rate: 2
Injection volume: 5
Detector: F ex 370 em 450

CHROMATOGRAM

Retention time: 17
Limit of detection: 0.3-1 fmole

OTHER SUBSTANCES

Simultaneous: arachidic acid, arachidonic acid, benzoic acid, butyric acid, capric acid, caproic acid, caprylic acid, deoxyuridine, glucuronic acid, imidazole-4-acetic acid, lauric acid, linoleic acid, linolenic acid, margaric acid, 1-methyl-4-imidazoleacetic acid, myristic acid, myristoleic acid, oleic acid, palmitic acid, palmitoleic acid, propionic acid, salicylic acid, stearic acid, thymidine, uridine, valeric acid

KEY WORDS

derivatization

REFERENCE

Yamaguchi,M.; Hara,S.; Matsunaga,R.; Nakamura,M.; Ohkura,Y. 3-Bromomethyl-6,7-dimethoxy-1-methyl-2(1H)-quinoxalinone as a new fluorescence derivatization reagent for carboxylic acids in high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *346*, 227–236.

SAMPLE
Matrix: solutions
Sample preparation: Prepare an aqueous solution, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:MeOH:buffer:triethylamine 4:4:92:0.01 (Buffer was 0.05% sodium octanesulfonate adjusted to pH 2.2 with 3 M phosphoric acid.)
Flow rate: 1.5
Injection volume: 10
Detector: UV 215

CHROMATOGRAM
Retention time: 9.5

OTHER SUBSTANCES
Simultaneous: benzaldehyde, benzoic acid, benzyl alcohol, protirelin

REFERENCE
Rao,G.N.; Sutherland,J.W.; Menon,G.N. High-performance liquid chromatographic assay for thyrotropin releasing hormone and benzyl alcohol in injectable formulation, *Pharm.Res.*, **1987**, *4*, 38–41.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Accubond Amino (J & W)
Mobile phase: MeCN:buffer 10 :90 (Buffer was 20 mM phosphoric acid adjusted to pH 3.0 with 20 mM NaOH.)
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 2.6

OTHER SUBSTANCES
Simultaneous: niacinamide, pyridoxal, pyridoxamine, thiamine, riboflavin, pyridoxine, vitamin B12

REFERENCE
J & W Catalog, 1992-3, p. 277.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 5 μL aliquot of a solution in MeCN:water 25:75.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:buffer 25:75 (Buffer was 2 mL glacial acetic acid and 700 mg 1-octanesulfonic acid in 750 mL water.)
Flow rate: 1
Injection volume: 5
Detector: UV 285

CHROMATOGRAM
Retention time: 4.7

OTHER SUBSTANCES
Simultaneous: flucytosine

REFERENCE
Wintermeyer,S.M.; Nahata,M.C. Stability of flucytosine in an extemporaneously compounded oral liquid, *Antimicrob.Agents Chemother.*, **1996**, *40*, 407–409.

SAMPLE
Matrix: sunscreen
Sample preparation: Weigh out 1 g sunscreen, add 2-10 mL mobile phase, stir magnetically for 5 min, filter (0.45 μm Millex-HV), inject an aliquot.

HPLC VARIABLES
Column: 200 × 5 5 μm Nucleosil C18
Mobile phase: MeCN:15 mM phosphoric acid 3:97 (55:45 for simultaneous determination of PABA esters and benzocaine)
Flow rate: 1
Injection volume: 20
Detector: UV 290

CHROMATOGRAM
Limit of detection: 500 ng/mL

REFERENCE
Bruze,M.; Gruvberger,B.; Thulin,I. PABA, benzocaine, and other PABA esters in sunscreens and after-sun products, *Photodermatol.Photoimmunol.Photomed.*, **1990**, *7*, 106–108.

SAMPLE
Matrix: urine
Sample preparation: 1 mL Urine + 1 mL 1 mg/mL m-hydroxybenzoic acid in 8 M NaOH, heat at 120° for 1 h. Remove a 10 μL aliquot and add it to 990 μL 50 mM phosphoric acid, centrifuge at 12000 rpm (Beckman Microfuge B), inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 10 μm Yanapak ODS-T C18 (Yamagimoto)
Mobile phase: MeCN:200 mM pH 3.5 potassium phosphate buffer 5:35
Column temperature: 55
Flow rate: 0.7
Injection volume: 10
Detector: E, Yanaco Model VMD-101, glassy carbon electrode +1.1 V

CHROMATOGRAM
Retention time: 7.5
Internal standard: m-hydroxybenzoic acid (9.5)

OTHER SUBSTANCES
Noninterfering: furosemide, metoclopramide, sulfamethoxazole, diazepam, oxazolam, clonidine, hydralazine, osalmid

KEY WORDS
p-aminohippuric acid cleaved to p-aminobenzoic acid under these conditions

REFERENCE
Ito,S.; Maruta,K.; Imai,Y.; Kato,T.; Ito,M.; Nakajima,S.; Fujita,K.; Kurahashi,T. Urinary p-aminobenzoic acid determined in the pancreatic function test by liquid chromatography, with electrochemical detection, *Clin.Chem.*, **1982**, *28*, 323–326.

SAMPLE
Matrix: urine
Sample preparation: Centrifuge, dilute 10-100 fold with water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeOH:10 mM tetrabutylammonium chloride, pH 7.4 10:90
Column temperature: 40
Flow rate: 1.4
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 5
Limit of detection: 60 ng/mL

OTHER SUBSTANCES
Extracted: p-aminohippuric acid, p-acetamidobenzoic acid, p-acetamidohippuric acid

REFERENCE
Karnes,H.T.; Riley,C.M.; Curry,S.H.; Schulman,S.G. Analysis of N-benzoyl-L-tyrosyl-p-aminobenzoic acid (bentiromide) metabolites in urine by ion-pair high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *338*, 377–388.

SAMPLE
Matrix: urine
Sample preparation: 100 μL Urine + 50 μL 0.1 mM (sic) pH 5.0 sodium acetate buffer + 20 μL β-glucuronidase (Helix pomatia), heat at 37° for 3 h, add 20 μL glacial acetic acid, add 50 μL 1 mg/mL 3,5-diaminobenzoic acid in MeOH, add 50 μL mobile phase, vortex for 30 s, centrifuge at 3000 rpm for 10 min, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb R, S5, ODS 2
Mobile phase: MeCN:buffer 3:97 (Buffer was 5 mM 1-heptanesulfonic acid in glacial acetic acid (Waters PIC-B7), pH 3.3.)
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 15
Internal standard: 3,5-diaminobenzoic acid (18)
Limit of quantitation: 2500 ng/mL

OTHER SUBSTANCES
Extracted: p-aminohippuric acid, p-acetamidohippuric acid, p-acetamidobenzoic acid

REFERENCE
Chan,K.; Miners,J.O.; Birkett,D.J. Direct and simultaneous high-performance liquid chromatographic assay for the determination of p-aminobenzoic acid and its conjugates in human urine, *J.Chromatogr.*, **1988**, *426*, 103–109.

SAMPLE
Matrix: urine
Sample preparation: 500 μL Urine diluted 1:10 + 50 μL MeOH + 100 μL 0.5 M HCl + 100 μL 0.1% sodium nitrite in water, vortex, let stand for 10 min, add 100 μL 2% ammonium sulfamate in water, let stand for 15 min, add 100 μL 0.05% 2-aminoanthracene

in MeCN (Caution! 2-Aminoanthracene causes cancer in experimental animals!), let stand for 15 min in the dark, add 5 mL diethyl ether, shake for 5 min, centrifuge. Remove 4 mL of the organic layer and evaporate it to dryness under vacuum, reconstitute the residue in 300 μL MeOH, inject a 30 μL aliquot.

HPLC VARIABLES

Column: 150 × 6 5 μm YMC-Pack A-312 (YMC)
Mobile phase: MeOH:water:acetic acid 78:22:1
Flow rate: 1
Injection volume: 30
Detector: UV 279

CHROMATOGRAM

Retention time: 20
Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Extracted: sulfanilamide, 4-aminobenzoyl-β-alanine

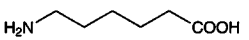
KEY WORDS

derivatization

REFERENCE

Hayashi,T.; Amino,M.; Uchida,G.; Sato,M. High-performance liquid chromatographic determination of primary aromatic amines in urine after derivatization to an azo dye with 2-aminoanthracene, *J.Chromatogr.B*, **1995**, *665*, 209–212.

Aminocaproic acid



Molecular formula: $C_6H_{13}NO_2$
Molecular weight: 131.17
CAS Registry No.: 60-32-2
Merck Index: 451

SAMPLE

Matrix: blood
Sample preparation: 20 μL Serum + 2 μL 3 mg/mL trans-4-aminomethylcyclohexanecarboxylic acid in water + 20 μL MeCN, mix, centrifuge at 10000 g for 3 min. Remove 5 μL of the supernatant and add it to 100 μL 25 mM pH 8 phosphate buffer, add 100 μL 300 μg/mL fluorescamine in acetone, vortex, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 10 μm LiChrosorb RP 18
Mobile phase: MeCN:water:acetic acid:THF 30:69:0.5:0.5, containing 40 mM sodium acetate
Flow rate: 2
Injection volume: 20
Detector: F ex 390 em 475

CHROMATOGRAM

Retention time: 4
Internal standard: tranexamic acid (trans-4-aminomethylcyclohexanecarboxylic acid) (5)
Limit of detection: 6000 ng/mL

KEY WORDS

serum; for epsilon-aminocaproic acid; derivatization

REFERENCE

Lacroix,C.; Levert,P.; Laine,G.; Goulle,J.P. Microdosage de deux antifibrinolytiques (acide β-aminocaproïque et acide tranexamique) par chromatographie liquide et détection fluorimétrique [Microanalysis of two antifibrinolytics (epsilon-aminocaproic acid and tranexamic acid) by liquid chromatography and fluorometry], *J.Chromatogr.*, **1984**, *309*, 183–186.

SAMPLE

Matrix: blood, urine
Sample preparation: 100 μL Plasma or urine + 10 μL 10% zinc sulfate, mix, add 100 μL MeOH, vortex, centrifuge for 1 min. Remove a 50 μL aliquot and add it to 300 μL IS solution, add 50 μL reagent, mix, after 1 min inject a 50 μL aliquot. (Prepare IS solution by adding 500 μL 1 mg/mL DL-valine to 20 mL 1 M pH 9.8 borate buffer. Prepare reagent by dissolving 20 mg o-phthalaldehyde and 24 mg N-acetyl-L-cysteine in 6 mL MeOH: water 50:50.)

HPLC VARIABLES

Column: 150 × 4.2 Nucleosil 5-C18
Mobile phase: MeCN:buffer 10:90 (Buffer was 10 g/L (?) ammonium acetate containing 5 mM L-proline and 2.5 mM copper sulfate.)
Flow rate: 2
Injection volume: 50
Detector: F

CHROMATOGRAM

Retention time: 7

Internal standard: valine (9 (L), 13 (D))
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Simultaneous: amino acids

KEY WORDS
derivatization; plasma

REFERENCE
Lam,S. High performance liquid chromatographic assay of Amicar, *epsilon*-aminocaproic acid, in plasma and urine after pre-column derivatization with *o*-phthalaldehyde for fluorescence detection, *Biomed.Chromatogr.*, **1990**, *4*, 175–177.

SAMPLE
Matrix: formulations
Sample preparation: Dissolve an amount of formulation containing 10-20 mg dexpanthenol in 10 mL 0.5 M HCl, heat at 85 ± 2° for 30 min to hydrolyze dexpanthenol to aminopropanol. Remove an aliquot containing 1-2 mg dexpanthenol and add it to 10 mL 0.4 mg/mL fluorescamine in MeCN, add 2 mL ϵ-aminocaproic acid (concentration 60% of that of dexpanthenol) in mobile phase, make up to 25 mL with mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 4.6 Chromegabond C18
Mobile phase: MeOH:100 mM borate buffer adjusted to pH 8.0 ± 0.1 with 2 M NaOH 30:70
Flow rate: 1
Injection volume: 20
Detector: F ex 390 em 475-490 or UV 390

CHROMATOGRAM
Retention time: 12
Internal standard: ϵ-aminocaproic acid

OTHER SUBSTANCES
Simultaneous: dexpanthenol

KEY WORDS
ϵ-aminocaproic acid is IS; derivatization

REFERENCE
Umagat,H.; Tscherne,R. High performance liquid chromatographic determination of panthenol in bulk, premix, and multivitamin preparations, *Anal.Chem.*, **1980**, *52*, 1368–1370.

SAMPLE
Matrix: formulations
Sample preparation: Tablets. Weigh out powdered tablet containing aminocaproic acid, dissolve in 100 mL water, filter (0.45 μm). Mix a 5 mL aliquot of the filtrate with 10 mL 5 mg/mL dansyl chloride in acetone and 10 mL 400 μg/mL tranexamic acid in buffer, let stand in the dark at room temperature for 30 min, add 2 drops ethanolamine, mix, let stand at room temperature for 15 min, make up to 50 mL with acetone:water 50:50, mix, inject an aliquot. Injections, syrup. Weigh out amount of injection or syrup containing 250 mg aminocaproic acid, dilute with 100 mL water, dilute an aliquot 5-fold with water. Mix a 5 mL aliquot with 10 mL 5 mg/mL dansyl chloride in acetone and 10 mL 400 μg/mL tranexamic acid in buffer, let stand in the dark at room temperature for 30 min, add 2 drops ethanolamine, mix, let stand at room temperature for 15 min, make up to 50 mL

with acetone:water 50:50, mix, inject an aliquot. (Prepare buffer by dissolving 550 mg anhydrous sodium carbonate in 300 mL water, add 300 mL acetone, mix.)

HPLC VARIABLES

Guard column: C18 (Alltech)
Column: 150 × 4.6 5 μm Econosphere C18
Mobile phase: MeOH:water:acetic acid:triethylamine 60:38:1.5:0.5
Flow rate: 1.5
Injection volume: 20
Detector: UV 335

CHROMATOGRAM

Retention time: 4.5
Internal standard: tranexamic acid (6.5)

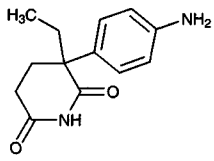
KEY WORDS

derivatization; tablets; injections; syrup

REFERENCE

Lau-Cam,C.A.; Roos,R.W. Assay of aminocaproic acid in dosage forms by reversed phase high performance liquid chromatography with dansylation, *J.Liq.Chromatogr.*, **1993**, *16*, 403–419.

Aminoglutethimide



Molecular formula: $C_{13}H_{16}N_2O_2$
Molecular weight: 232.28
CAS Registry No.: 125-84-8
Merck Index: 460
Lednicer No.: 1 257

SAMPLE

Matrix: blood, saliva, urine
Sample preparation: 300 μL Plasma, urine, or saliva + 150 μL 50 μg/mL IS in MeOH + 300 μL 100 mM pH 5.6 acetate buffer + 5 mL dichloromethane, vortex for 1 min, centrifuge at 1760 g for 15 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 300 μL MeOH, vortex for 1 min, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: Guard-Pak Resolve silica (Waters)
Column: two Chiralcel OD cellulose tris(3,5-dimethylphenyl)carbamate columns in series
Mobile phase: Hexane:MeOH:isopropanol 65:17.5:17.5 0.7
Flow rate: 0.7
Injection volume: 50
Detector: UV 245

CHROMATOGRAM

Retention time: 32.65 (R), 43.66 (S)
Internal standard: (4'-aminophenyl)-3-methyl-1-methylpyrrolidine-2,5-dione (one enantiomer only) (37.74)
Limit of detection: 320 ng/mL

OTHER SUBSTANCES

Extracted: metabolites
Simultaneous: acetaminophen, carmustine, codeine, dexamethasone, hydrocortisone, ibuprofen, mitomycin, morphine, prednisone, tamoxifen, vincristine
Noninterfering: aspirin, busulfan, cyclophosphamide, indomethacin, methotrexate, vincristine

KEY WORDS

plasma; chiral

REFERENCE

Alshowaier,I.A.; el-Yazigi,A.; Ezzat,A.; El-Warith,A.E.; Nicholls,P.J. Liquid chromatographic separation and measurement of optical isomers of aminoglutethimide and its acetyl metabolite in plasma, saliva, and urine, *Ther.Drug Monit.*, **1995**, *17*, 538–543.

SAMPLE

Matrix: bulk
Sample preparation: Dissolve in mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 10 μm Chiralcel OJ
Mobile phase: EtOH:hexane 80:20
Flow rate: 1
Injection volume: 20
Detector: UV 365

CHROMATOGRAM
Retention time: k' 2.00 ((R)-(+)), k' 5.14 ((S)-(-))

KEY WORDS
chiral

REFERENCE
Francotte,E.R.; Richert,P. Applications of simulated moving-bed chromatography to the separation of the enantiomers of chiral drugs, *J.Chromatogr.A*, **1997**, *769*, 101–107.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out amount of finely powdered tablet corresponding to 20.83 mg aminoglutethimide, add 1 mL EtOH, add 5 mL water, sonicate for 30 min, make up to 10 mL with water, filter, discard the first 2 mL filtrate. Remove a 40 μL aliquot of the filtrate and add it to 500 μL pH 4.5 phosphate buffer, add 300 μL 2.6 mg/mL 1,2-naphthoquinone-4-sulfonic acid in water (prepare fresh each day), heat at 60° for 15 min, cool, add 4 mL n-butanol:ethyl acetate 2:1, shake vigorously for 30 s, centrifuge at 1750 rpm for 10 min. Remove a 100 μL aliquot of the organic layer and add it to 250 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water 90:10
Flow rate: 0.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 5.53
Limit of detection: 50 ng

KEY WORDS
derivatization; tablets

REFERENCE
Ozkirimli,S.; Sevingil,M. High pressure liquid chromatographic determination of aminoglutethimide, *Acta Pharm.Turc.*, **1989**, *31*, 57–60.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 62 × 2 packed with chiral packing (Prepare packing by dissolving 4-chloro-3-methylphenylcarbamate cellulose in THF, coat on Nucleosil 1000-7, dry at 60° for 3 h under reduced pressure.)
Mobile phase: Hexane:isopropanol:diethylamine 80:20:0.1
Flow rate: 0.1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 25.00

KEY WORDS
narrow-bore; chiral; α 1.20

REFERENCE

Chankvetadze,B.; Chankvetadze,L.; Sidamonidze,S.; Yashima,E.; Okamoto,Y. Enantioseparation of some chiral pharmaceuticals using narrow-bore liquid chromatography, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 695–699.

SAMPLE

Matrix: solutions
Sample preparation: Inject an aliquot of a 100 μg/mL solution in mobile phase.

HPLC VARIABLES

Column: 150 × 4 5 μm Crownpak CR(+) immobilized crown ether
Mobile phase: MeOH:0.1% pH 1.9 perchloric acid 15:85
Column temperature: 25
Flow rate: 1
Detector: UV 210

CHROMATOGRAM

Retention time: 10.08, 11.05

KEY WORDS

chiral; comparison with capillary electrophoresis

REFERENCE

Nishi,H.; Nakamura,K.; Nakai,H.; Sato,T. Separation of enantiomers and isomers of amino compounds by capillary electrophoresis and high-performance liquid chromatography utilizing crown ethers, *J.Chromatogr.A*, **1997**, *757*, 225–235.

SAMPLE

Matrix: urine
Sample preparation: 50 mL urine + 50 mL water, extract three times with 25 mL dichloromethane. Combine the organic layers and concentrate them under reduced pressure, evaporate to dryness under a stream of nitrogen, reconstitute the residue in MeOH, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 10 μm Chiralcel OD + 250 × 4.6 10 μm Chiralcel OJ (Daicel)
Mobile phase: Hexane:isopropanol 50:50
Flow rate: 0.7
Detector: UV 257

CHROMATOGRAM

Retention time: 32.0 ((-)-S), 53.1 ((+)-R)

OTHER SUBSTANCES

Extracted: acetylaminoglutethimide

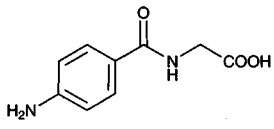
KEY WORDS

chiral

REFERENCE

Aboul-Enein,H.Y.; Islam,M.R. Direct enantiomeric high performance liquid chromatographic separation of aminoglutethimide and its major metabolite on a series of Chiralcel OD and Chiralcel OJ columns and its application to biological fluids, *Biomed.Chromatogr.*, **1991**, *5*, 74–77.

Aminohippuric acid



Molecular formula: $C_9H_{10}N_2O_3$
Molecular weight: 194.19
CAS Registry No.: 61-78-9, 94-16-6 (sodium salt)
Merck Index: 462

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma or urine + 0.5-5 μg p-aminobenzoic acid + 200 μL MeCN, vortex for a few s, centrifuge at 800 g for 5 min, inject a 5 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:0.04% pH 2.5 ± 0.05 phosphoric acid 3.5:96.5
Flow rate: 1.5
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 4.5
Internal standard: p-aminobenzoic acid (8)
Limit of detection: 1 μg/mL

OTHER SUBSTANCES
Extracted: iothalamate

KEY WORDS
plasma; dog; human; pharmacokinetics

REFERENCE
Prueksaritanont,T.; Chen,M.L.; Chiou,W.L. Simple and micro high-performance liquid chromatographic method for simultaneous determination of p-aminohippuric acid and iothalamate in biological fluids, *J.Chromatogr.*, **1984**, *306*, 89–97.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Serum + 50 μL 20 μg/mL p-aminobenzoic acid in MeCN, vortex for 15 s, centrifuge at 10000 g for 5 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 100 × 8 10 μm μBondapak C18
Mobile phase: 67 mM pH 2.8 phosphate buffer containing 5 mM tetrabutylammonium phosphate (low-UV Pic A Reagent, Waters) (Buffer was KH_2PO_4:Na_2HPO_4 97.5:2.5.)
Flow rate: 2
Injection volume: 20
Detector: UV 275

CHROMATOGRAM
Retention time: 7.4
Internal standard: p-aminobenzoic acid (13.0)
Limit of detection: 1000 ng/mL

KEY WORDS
serum

REFERENCE
Jenny,P.D.; Weber,A.; Smith,A.L. Quantitation of p-aminohippuric acid in biological fluids by high-performance liquid chromatography and dual-wavelength ultraviolet detection, *J.Chromatogr.*, **1989**, *490*, 213–218.

SAMPLE
Matrix: blood
Sample preparation: Add barbital to plasma. 100 μL Plasma + 500 μL MeOH, vortex for 15 s, centrifuge at 2500 rpm for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in buffer, inject a 15-20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Octyl C8 (Rainin)
Mobile phase: MeOH:MeCN:buffer 90:10:300 (Buffer was 6.44 g KH_2PO_4, 7.04 g K_2HPO_4, and 14 mL 500 mM dodecyltriethylammonium phosphate (Regis) in 4 L water.)
Flow rate: 1
Injection volume: 15-20
Detector: UV 254

CHROMATOGRAM
Retention time: 12.0
Internal standard: barbital (15.9)
Limit of quantitation: 5000 ng/mL

OTHER SUBSTANCES
Extracted: iothalamic acid

KEY WORDS
plasma

REFERENCE
Jayewardene,A.L.; Seneviratne,A.K.; Gambertoglio,J.G. Paired ion reversed-phase HPLC assay for the simultaneous determination of iothalamic acid and para aminohippuric acid in plasma, *J.Liq.Chromatogr.*, **1994**, *17*, 2395–2412.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Serum + 50 μL 250 μg/mL acetaminophen in 100 mM HCl, add to SPE cartridge containing 150 mg 80-100 mesh Chromosorb P/NAW, elute with 1 mL ethyl acetate:MeOH 5:1, add the eluate to 50 μL 100 mM HCl, vortex for 15 s, centrifuge at 10000 g for 3 min, inject a 20 μL aliquot of the lower aqueous phase.

HPLC VARIABLES
Column: 5 μm C8
Mobile phase: MeCN:20 mM pH 3.3 phosphoric acid 2.5:97.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Internal standard: acetaminophen
Limit of detection: <1 μg/mL

OTHER SUBSTANCES
Extracted: iohexol

KEY WORDS
serum; SPE

REFERENCE
Andreeva,M.; Rapondjieva,A.; Deskova,D.; Tishkov,I.; Svinarov,D. Liquid chromatographic determination of iohexol and PAH with Chromosorb P column used for sample preparation (Abstract 175), *Ther.Drug Monit.*, **1995**, *17*, 427–427.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma + 150 μL salicylic acid in 1 M perchloric acid, centrifuge at 10000 g for 5 min, inject a 30 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.5 5 μm Cosmosil MS-C18
Mobile phase: MeCN:water:glacial acetic acid 3:100:1, adjusted to pH 4.0 with 10 M NaOH
Column temperature: 40
Flow rate: 1.5
Injection volume: 30
Detector: F ex 270 em 350

CHROMATOGRAM
Retention time: 4.01
Internal standard: salicylic acid (17)
Limit of detection: 50 ng/mL
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Extracted: p-acetamidobenzoic acid, p-acetamidohippuric acid, p-aminobenzoic acid

KEY WORDS
plasma; rabbit; pharmacokinetics

REFERENCE
Song,D.J.; Hsu,K.Y. Determination of p-aminobenzoic acid and its metabolites in rabbit plasma by high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.B*, **1996**, *677*, 69–75.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. Mix 100 μL plasma with 111 μg/mL 4-acetamidobenzoic acid in MeCN:water 10:90, vortex, add 25 μL 20% trichloroacetic acid, vortex, centrifuge at 10000 g for 10 min, inject a 25 μL aliquot. Urine. Mix 100 μL urine (diluted 1:50 with water) with 25 μL 333 μg/mL 4-acetamidobenzoic acid in MeCN:water 1:29, vortex, add 25 μL 20% trichloroacetic acid, vortex, centrifuge at 10 000 g for 10 min, inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: 8 × 4 5 μm Nucleosil 100 C18 AB ChromCart
Column: 125 × 4 5 μm Nucleosil 100 C18 AB ChromCart
Mobile phase: Gradient. MeOH:pH 3.9 buffer from 1:99 to 15:85 over 15 min. (pH 3.9 Buffer was 375 ml solution C containing 1.0 g sodium heptanesulfonate monohydrate and 2.7 mL 85% orthophosphoric acid made up to1000 mL with water. Solution C was 21.01 g citric acid monohydrate and 8.0 g sodium hydroxide in 1000 mL water.)
Flow rate: 1

Injection volume: 25
Detector: UV 273 for 5 min, UV 265 for 15 min

CHROMATOGRAM
Retention time: 3.6
Internal standard: 4-acetamidobenzoic acid (12.3)
Limit of detection: 200 ng/mL (plasma)
Limit of quantitation: 2.5 μg/mL (plasma)

OTHER SUBSTANCES
Extracted: metabolite

KEY WORDS
pharmacokinetics; plasma

REFERENCE
Decosterd,L.A.; Karagiannis,A.; Roulet,J.-M.; Bélaz,N.; Appenzeller,M.; Buclin,T.; Vogel,P.; Biollaz,J. High-performance liquid chromatography of the renal blood flow marker p-aminohippuric acid (PAH) and its metabolite N-acetyl PAH improves PAH clearance methods, *J.Chromatogr.B*, **1997**, *703*, 25–36.

SAMPLE
Matrix: blood, urine
Sample preparation: Dilute urine 10-fold with water. Add 200 μL MeCN containing 20 μg/mL p-aminobenzoic acid to 100 μL diluted urine, vortex briefly, centrifuge at 12000 g for 4 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 5 μm Ultrasphere C18
Column: 250 mm 5 μm Primesphere C18 (Torrance, USA)
Mobile phase: MeOH:buffer 18:82 (Buffer was 50 mM NaH_2PO_4 with 0.5 mM tetrabutyl ammonium hydrogen sulfate with an unadjusted pH of 4.11.)
Flow rate: 0.8
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 6.2
Internal standard: p-aminobenzoic acid (9.8)

OTHER SUBSTANCES
Extracted: iothalamic acid

KEY WORDS
serum

REFERENCE
Agarwal,R. Chromatographic estimation of iothalamate and p-aminohippuric acid to measure glomerular filtration rate and effective renal plasma flow in humans, *J.Chromatogr.B*, **1998**, *705*, 3–9.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 500 μL Plasma + 500 μL 1 M HCl, vortex for 10 s, add 6 mL ethyl acetate, vortex for 20 s, centrifuge at 4° at 1700 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 400 μL 25 mM pH 3 KH_2PO_4, add 500 μL dichloromethane, shake gently horizontally for 5 min, centrifuge at 1700 g for 5 min, inject a 20 μL aliquot of the aqueous phase. Urine.

Dilute 1:10 with water. Remove a 1 mL aliquot and add it to 1 mL 1 M HCl, vortex for 10 s, add 6 mL ethyl acetate, vortex for 20 s, centrifuge at 4° at 1700 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 400 μL 25 mM pH 3 KH_2PO_4, add 500 μL dichloromethane, shake gently horizontally for 5 min, centrifuge at 1700 g for 5 min, inject a 20 μL aliquot of the aqueous phase.

HPLC VARIABLES
Guard column: 10 × 3 anion-exchange guard column (Chrompack)
Column: 250 × 4.6 Partisil 10 SAX
Mobile phase: MeCN:25 mM pH 3 phosphate buffer 15:85
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 4

OTHER SUBSTANCES
Extracted: iothalamate
Noninterfering: acipimox, allopurinol, aspirin, atenolol, captopril, chlorthalidone, clonidine, digitoxin, digoxin, diltiazem, dipyridamole, enalapril, furosemide, gemfibrozil, hydralazine, hydrochlorothiazide, ibopamine, insulin, inulin, isosorbide dinitrate, α-methyldopa, nicardipine, nifedipine, prazosin, propranolol, salicylic acid, simvastatin, trinitrin, verapamil

KEY WORDS
plasma

REFERENCE
Gaspari,F.; Mainardi,L.; Ruggenenti,P.; Remuzzi,G. High-performance liquid chromatographic determination of iothalamic acid in human plasma and urine, *J.Chromatogr.*, **1991**, *570*, 435–440.

SAMPLE
Matrix: perfusate
Sample preparation: Adjust pH of 5-10 mL perfusate to 5 with 180 μL 2.5 M HCl, extract twice with an equal volume of ethyl acetate. Combine the organic layers, add 1 mL water, evaporate them to 1 mL under vacuum, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water containing 30 μL/L triethylamine, adjusted to pH 2.3 with phosphoric acid 10:90
Flow rate: 1.5
Injection volume: 20
Detector: UV 290

CHROMATOGRAM
Retention time: 3.0

OTHER SUBSTANCES
Extracted: procaine, aminobenzoic acid, 4-acetamidobenzoic acid

KEY WORDS
rabbit; chinchilla; pharmacokinetics

REFERENCE
Henrikus,B.M.; Kampffmeyer,H.G. Ester hydrolysis and conjugation reactions in intact skin and skin homogenate, and by liver esterase of rabbits, *Xenobiotica*, **1992**, *22*, 1357–1366.

SAMPLE
Matrix: urine
Sample preparation: Centrifuge, dilute 10-100 fold with water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeOH:10 mM tetrabutylammonium chloride, pH 7.4 10:90
Column temperature: 40
Flow rate: 1.4
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 7
Limit of detection: 120 ng/mL

OTHER SUBSTANCES
Extracted: p-aminobenzoic acid, p-acetamidobenzoic acid, p-acetamidohippuric acid

REFERENCE
Karnes,H.T.; Riley,C.M.; Curry,S.H.; Schulman,S.G. Analysis of N-benzoyl-L-tyrosyl-p-aminobenzoic acid (bentiromide) metabolites in urine by ion-pair high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *338*, 377–388.

SAMPLE
Matrix: urine
Sample preparation: 100 μL Urine + 50 μL 0.1 mM (sic) pH 5.0 sodium acetate buffer + 20 μL β-glucuronidase (Helix pomatia), heat at 37° for 3 h, add 20 μL glacial acetic acid, add 50 μL 1 mg/mL 3,5-diaminobenzoic acid in MeOH, add 50 μL mobile phase, vortex for 30 s, centrifuge at 3000 rpm for 10 min, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb R, S5, ODS 2
Mobile phase: MeCN:buffer 3:97 (Buffer was 5 mM 1-heptanesulfonic acid in glacial acetic acid (Waters PIC-B7), pH 3.3.)
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 11.8
Internal standard: 3,5-diaminobenzoic acid (18)
Limit of quantitation: 2500 ng/mL

OTHER SUBSTANCES
Extracted: p-aminobenzoic acid, p-acetamidohippuric acid, p-acetamidobenzoic acid

REFERENCE
Chan,K.; Miners,J.O.; Birkett,D.J. Direct and simultaneous high-performance liquid chromatographic assay for the determination of p-aminobenzoic acid and its conjugates in human urine, *J.Chromatogr.*, **1988**, *426*, 103–109.

SAMPLE

Matrix: urine

Sample preparation: Dilute urine 1:100 or 1:500. 200 μL Diluted urine + 50 μL barbital solution, vortex for 15 s, inject a 20-30 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultrasphere C8

Mobile phase: MeCN:MeOH:10 mM pH 7.5 potassium phosphate buffer:0.5 M dodecyl triethylammonium phosphate 6:94:300:0.6 (0.5 M Dodecyl triethylammonium phosphate was Q-12, Ion pair reagent, Regis Chemical Co.)

Flow rate: 1

Injection volume: 20-30

Detector: UV 254

CHROMATOGRAM

Retention time: 10.4

Internal standard: barbital (14.5)

Limit of quantitation: 75000 ng/mL

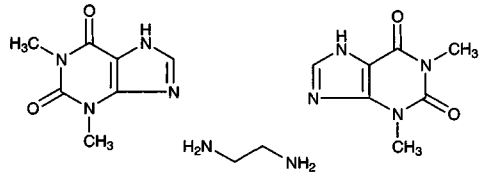
OTHER SUBSTANCES

Extracted: iothalamate

REFERENCE

Seneviratne,A.K.; Jayewardene,A.L.; Gambertoglio,J.G. Paired-ion reversed-phase HPLC assay for the determination of iothalamic acid and para aminohippuric acid in urine, *J.Pharm.Biomed.Anal.*, **1994**, *12*, 1311–1316.

Aminophylline



Molecular formula: $C_{16}H_{24}N_{10}O_4$
Molecular weight: 420.43
CAS Registry No.: 317-34-0
Merck Index: 485
Lednicer No.: 1 427

SAMPLE

Matrix: formulations
Sample preparation: Add 10 mL acetone:water 50:50 to 100-400 mg cream, stir for several min. Add 10 mL chloroform (Caution! Chloroform is a carcinogen!), shake for 2 min, centrifuge at 3000 rpm for 5 min, remove the organic layer. Mix a 1-5 mL aliquot of the organic layer with 10 mL 5 mg/mL dansyl chloride in acetone and 10 mL 900 mg/mL sodium carbonate in acetone:water 50:50. Let the mixture stand at room temperature in the dark for 12 h. Make up to 50 mL. Inject a 10 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Microsorb MV C18
Mobile phase: MeOH:water:acetic acid:triethylamine 69:29:1.5:0.5
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 7.01 (dansyl-theophylline), 9.21 (bis-dansyl-ethylenediamine)

KEY WORDS

derivatization; cream

REFERENCE

Haky,J.E.; Foss,W.M.; Marks,B.L. Analysis of aminophylline in thigh cream formulations by high performance liquid chromatography, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 2399–2414.

SAMPLE

Matrix: formulations
Sample preparation: Tablets. Weigh out powdered tablets containing 100 mg aminophylline, add 50 mL water, sonicate for 15 min, make up to 100 mL with water, mix, filter. Remove a 5 mL aliquot of the filtrate and add it to 10 mL 5 mg/mL dansyl chloride in acetone and 5 mL buffer, mix gently, let stand in the dark for 12 h, make up to 50 mL with acetone:water 50:50, mix, inject an aliquot. Injections, oral liquids. Measure out an amount containing 100 mg aminophylline, make up to 100 mL with water, mix. Remove a 5 mL aliquot and add it to 10 mL 5 mg/mL dansyl chloride in acetone and 5 mL buffer, mix gently, let stand in the dark for 12 h, make up to 50 mL with acetone:water 50:50, mix, inject an aliquot. (Prepare buffer by dissolving 550 mg anhydrous sodium carbonate in 300 mL water, add 300 mL acetone, mix.)

HPLC VARIABLES

Guard column: 70 × 2.1 Co:Pell ODS
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water:acetic acid:triethylamine 60:38:1.5:0.5 (A) or 65:33:1.5:0.5 (B)
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 6.0 (theophylline (mobile phase B)), 7.3 (ethylenediamine (mobile phase B)), 7.45 (theophylline (mobile phase A)), 12.00 (ethylenediamine (mobile phase A))

KEY WORDS

derivatization; tablets; injections; oral solutions

REFERENCE

Lau-Cam,C.A.; Roos,R.W. Simultaneous high performance liquid chromatographic determination of theophylline and ethylenediamine in aminophylline dosage forms as their dansyl derivatives, *J.Liq.Chromatogr.*, **1991**, *14*, 1939–1956.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

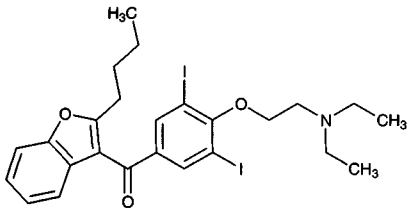
Also analyzed: acepromazine, acetaminophen, acetophenazine, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine,

phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Amiodarone



Molecular formula: $C_{25}H_{29}I_2NO_3$
Molecular weight: 645.32
CAS Registry No.: 1951-25-3
Merck Index: 504
Lednicer No.: 4 127, 156

SAMPLE

Matrix: blood
Sample preparation: Precipitate 100 µL serum with 200 µL 5 µg/mL IS in MeCN, centrifuge at 12 000 g for 5 min. Inject a 50 µL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 4 × 4 5 µm LiChroCART LiChrospher 60 RP Select B
Column: 125 × 4 5 µm LiChroCART LiChrospher 60 RP Select B
Mobile phase: MeCN:buffer 10:90 (Buffer was 25 mM pH 3.0 triethylammonium phosphate containing 2% MeCN.)
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: 4.58
Internal standard: promazine (3.39)
Limit of detection: 90 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

serum

REFERENCE

Hannak,D.; Scharbert,F.; Kattermann,R. Stepwise binary gradient high-performance liquid chromatographic system for routine drug monitoring, *J.Chromatogr.A*, **1996**, *728*, 307–310.

SAMPLE

Matrix: blood
Sample preparation: Condition a 1 mL cyano-bonded silica (J.T.Baker) SPE cartridge with 1 mL MeOH and three 1 mL portions of water. Mix 100 µL serum with 100 µL 2 µg/mL IS in MeOH:water 70:30 and 500 µL water. Add to the SPE cartridge, allow to pass through under gravity. Wash three times with 1 mL portions of water and with 1 mL MeOH:water 50:50. Elute with 1 mL MeOH containing 1 mL/L triethylamine. Evaporate the eluate under a stream of nitrogen at 40°, reconstitute the residue in 100 µL mobile phase. Inject a 20 µL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 µm Zorbax cyano bonded silica
Mobile phase: MeCN:MeOH:phosphate buffer 23:37:40, adjusted to pH 3.5
Column temperature: 45
Flow rate: 0.8
Injection volume: 20
Detector: UV 241

CHROMATOGRAM
Retention time: 7.92
Internal standard: tamoxifen (5.57)
Limit of detection: 5 ng

OTHER SUBSTANCES
Extracted: desethylamiodarone, bepridil, L8040 (Sanofi Recherche), trifluoperazine
Simultaneous: aprindine, bromocriptine, captopril, carbamazepine, chlorpromazine, diltiazem, dimefline, dipyridamole, disopyramide, flecainide, flurazepam, furosemide, imipramine, labetalol, miconazole, nifedipine, norverapamil, procainamide, propafenone, propranolol, quinidine, tocainide, trifluoropromazine, verapamil, warfarin

KEY WORDS
serum; SPE

REFERENCE
Pollak,P.T. A systematic review and critical comparison of internal standards for the routine liquid chromatographic assay of amiodarone and desethylamiodarone, *Ther.Drug Monit.*, **1996**, *18*, 168–178.

SAMPLE
Matrix: blood
Sample preparation: Mix 200 µL serum with 20 µL 25 µg/mL IS in MeOH and 100 µL 500 mM KH_2PO_4. Add 4 mL hexane, shake for 3 min, centrifuge, freeze at -20°. Evaporate the organic layer to dryness under a stream of argon in a 37° water bath, reconstitute in 100 µL MeOH, inject a 50 µL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 5 µm Supelguard LC-CN
Column: 150 × 4.6 5 µm Supelcosil LC-CN
Mobile phase: MeCN:MeOH:water: 500 mM KH_2PO_4 13.6:48:36:2.4
Flow rate: 1.5
Injection volume: 50
Detector: UV 240

CHROMATOGRAM
Retention time: 6.7
Internal standard: L8040 (8.0)
Limit of detection: 10 ng/mL
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Extracted: metabolite
Noninterfering: acebutolol, aprobarbital, atenolol, bupranolol, celiprolol, clobazam, debrisoquine, diazepam, diltiazem, flecainide, gallopamil, hexobarbiltal, lidocaine, mephenytion, metopolol, mexiletine, nadolol, pentobarbital, phenacetin, prazosin, procainamide, progesterone, propafenone, propranolol, quinidine, sotalol, theophylline, verapamil

KEY WORDS
serum

REFERENCE
Kunicki,P.K.; Sitkiewicz,D. High performance liquid chromatographic analysis of some antiarrhythmic drugs in human serum using cyanopropyl derivatized silica phase, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1169–1181.

SAMPLE
Matrix: blood

Sample preparation: 500 μL Serum + 250 μL di-iso-propyl ether:n-butyl alcohol 7:3 containing 800 ng/mL minaprine, centrifuge 2 min, shake, centrifuge 5 min, inject 50 μL aliquot of top organic layer.

HPLC VARIABLES

Guard column: 30 × 4.6 5 μm Brownlee cyano spheri-5
Column: 250 × 4.6 5 μm Altex ultrasphere cyano
Mobile phase: MeCN:THF:water:2 M ammonium formate (pH 4.0) 700:100:195:5
Column temperature: 20
Flow rate: 1.5
Injection volume: 50
Detector: UV 242

CHROMATOGRAM

Retention time: 8
Internal standard: minaprine (5.5)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES

Simultaneous: propafenone, desethylamiodarone, diltiazem, verapamil, nortriptyline, amitriptyline
Also analyzed: haloperidol, desipramine, imipramine, clomipramine

KEY WORDS

serum

REFERENCE

Mazzi,G. Simple and practical high-performance liquid chromatographic assay of some tricyclic drugs, haloperidol, diltiazem, verapamil, propafenone, and amiodarone, *Chromatographia*, **1987**, *24*, 313–316.

SAMPLE

Matrix: blood
Sample preparation: Condition a 1 mL 100 mg Phenomenex C2 SPE cartridge (cat. no. AHO-0857) with 1 mL MeOH then 1 mL water (add analyte within 2-3 min). 500 μL Serum + 300 μL 2 M pH 4.5 sodium acetate + 10 μL 100 μg/mL triflupromazine in water, vortex for 5-10 s, add to SPE cartridge, wash with 1 mL water, 1 mL MeOH:water 1:1, 1 mL MeCN:water 1:1, elute with 500 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 2 Vydac C18 reverse phase (cat. no. 201SC)
Column: 150 × 4.6 5 μm UltraCarb 5 octadecylsilyl (cat. no. OOF-0351-EO)
Mobile phase: 875 mL MeOH:MeCN 1:1 + 125 mL 30 mM pH 4.0 ammonium acetate
Column temperature: 40
Flow rate: 1.5
Injection volume: 50
Detector: UV 242

CHROMATOGRAM

Retention time: 6.5
Internal standard: triflupromazine (2.2)
Limit of quantitation: 160 ng/mL

OTHER SUBSTANCES

Simultaneous: metabolites
Noninterfering: acetaminophen, N-acetylprocainamide, amikacin, amitriptyline, caffeine, carbamazepine, chloramphenicol, clonazepam, cyclosporine, desipramine, digoxin, disopyramide, ethosuximide, flecainide, gentamicin, haloperidol, imipramine, kanamycin, li-

docvaine, methotrexate, netilmicin, nortriptyline, phenobarbital, phenytoin, primidone, procainamide, propranolol, propoxyphene, quinidine, salicylic acid, streptomycin, theophylline, tobramycin, valproic acid, vancomycin

KEY WORDS
serum; SPE

REFERENCE
Jandreski,M.A.; Vanderslice,W.E. Clinical measurement of serum amiodarone and desethylamiodarone by using solid-phase extraction followed by HPLC with a high-carbon reversed-phase column, *Clin.Chem.*, **1993**, *39*, 496–500.

SAMPLE
Matrix: blood
Sample preparation: Make serum alkaline with 500 mM KH_2PO_4, extract with hexane.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil LC-CN
Mobile phase: MeCN:MeOH:water:500 mM KH_2PO_4 13.6:48:36:2.4
Flow rate: 1.5
Detector: UV 240

CHROMATOGRAM
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum

REFERENCE
Kunicki,P.K.; Sitkiewicz,D. High-performance liquid chromatographic determination of some antiarrhythmic drugs using cyanopropyl derivatized silica phase (Abstract 43), *Ther.Drug Monit.*, **1995**, *17*, 394–394.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 500 μL water + 500 μL 2 M pH 3.1 acetate buffer + 50 μL 32.4 μM IS in meOH + 6 mL dichloromethane, extract. Remove the lower organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH, inject a 15 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 4 μm Nova-Pak C18
Mobile phase: MeOH:water:concentrated (25%) ammonia 99.45:3:0.55
Flow rate: 1.1
Injection volume: 15
Detector: UV 254

CHROMATOGRAM
Retention time: 4
Internal standard: L8040 (6)

OTHER SUBSTANCES
Extracted: metabolites, desethylamiodarone

KEY WORDS
serum; comparison with capillary electrophoresis

REFERENCE
Zhang,C.-X.; Aebi,Y.; Thormann,W. Microassay of amiodarone and desethylamiodarone in serum by capillary electrophoresis with head-column field-amplified sample stacking, *Clin.Chem.*, **1996**, *42*, 1805–1811.

SAMPLE
Matrix: blood, tissue
Sample preparation: Caution! Isopropyl ether may form explosive peroxides! Serum. 250 μL Serum + 100 μL 360 mM NaH_2PO_4 + 100 μL 6 μg/mL IS in MeOH + 200 μL isopropyl ether, vortex for 30 s, centrifuge at 3000 g for 3 min, inject a 50 μL aliquot of the organic layer. Tissue. Pound into a thin layer, freeze, lyophilize, pulverize, extract into isopropyl ether:MeOH 1:1, add IS, inject an aliquot.

HPLC VARIABLES
Column: 150 × 3.9 Resolve 5 μm spherical silica (Waters)
Mobile phase: MeOH:buffer 92:8 (Buffer was 2.2 g ammonium sulfate in 1 L water adjusted to pH 6.8 with 1 M NaOH.)
Flow rate: 1.8
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 5.7
Internal standard: 2-ethyl-3-(3,5-dibromo-4γ-dipropylaminopropoxybenzoyl)benzothiophene (L8040) (4.4)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
serum; normal phase; myocardium; lung; adipose; muscle

REFERENCE
Ou,C.-N.; Rognerud,C.L.; Duong,L.T.; Frawley,V.L. Liquid-chromatographic determination of amiodarone and N-desethylamiodarone in serum, *Clin.Chem.*, **1990**, *36*, 532–534.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 204

CHROMATOGRAM
Retention time: 21.915

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: incubations
Sample preparation: 500 μL Incubation medium + 500 μL 100 mM pH 6.5 phosphate buffer + 5 mL hexane, shake for 10 min, repeat extraction. Combine the organic layers and evaporate them to dryness at 30° under a stream of nitrogen, reconstitute in 1 mL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 mm long 5 μm Spherisorb CN
Mobile phase: Hexane:isopropanol:sulfuric acid 49.98:49.98:0.04
Flow rate: 1.5
Detector: UV 242

CHROMATOGRAM
Retention time: 8.8
Limit of detection: 10 ng

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
rat; rabbit; normal phase; incubations

REFERENCE
Young,R.A.; Mehendale,H.M. In vitro metabolism of amiodarone by rabbit and rat liver and small intestine, *Drug Metab.Dispos.*, **1986**, *14*, 423–429.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Add 1.25 μg/mL IS in MeCN to microsomal incubation, mix vigorously for 30 s, centrifuge at 1500 g for 5 min, inject a 250 μL aliquot of the supernatant. (Use amber tubes.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm ODS Hypersil C18
Mobile phase: MeOH:water:58% ammonium hydroxide 88:10:2
Flow rate: 1.8
Injection volume: 250
Detector: UV 242

CHROMATOGRAM
Retention time: 8.1
Internal standard: 2-ethyl-3-(3,5-dibromo-4γ-dipropylaminopropoxybenzoyl)benzothiophene (L8040) (13.5)
Limit of detection: 50 nM

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
rat

REFERENCE
Trivier,J.M.; Pommery,J.; Libersa,C.; Caron,J.; Lhermitte,M. High-performance liquid chromatographic assay for amiodarone N-deethylation in microsomes of rat liver, *J.Chromatogr.*, **1992**, *579*, 269–276.

SAMPLE
Matrix: perfusate
Sample preparation: Centrifuge intestinal perfusate at 3000 rpm for 15 min, inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μ Bondapak C18
Mobile phase: MeOH:10 mM pH 3.0 phosphoric acid 83:17
Flow rate: 2
Injection volume: 10
Detector: UV 242

KEY WORDS
rat; small intestine

REFERENCE
Martín-Algarra,R.V.; Pascual-Costa,R.M.; Merino,M.; Casabo,V.G. Intestinal absorption kinetics of amiodarone in rat small intestine, *Biopharm.Drug Dispos.*, **1997**, *18*, 523–532.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 10 μm LiChrosorb
Mobile phase: MeCN:MeOH:buffer 3:6:1 (Buffer was 67 mM KH_2PO_4 adjusted to pH 2.9 with phosphoric acid.)
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 7.46

OTHER SUBSTANCES
Simultaneous: flecainide acetate

REFERENCE
Paw,B.; Przyborowski,L.; Slawik,T. Determination of flecainide acetate in tablets by HPLC and UV-spectrophotometry, *Pharmazie*, **1998**, *53*, 97–98.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica

Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

Flow rate: 2

Injection volume: 20

Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 3.0

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions
Sample preparation: Inject a 50 μL aliquot of a solution in MeOH.

HPLC VARIABLES

Column: 200 × 4 10 μm LiChrosorb RP-18
Mobile phase: MeCN:MeOH:pH 2.50 phosphate buffer 15:80:5
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: 6.2

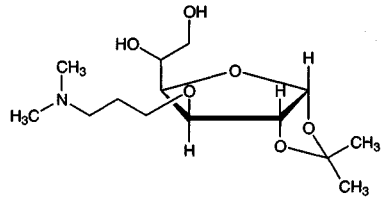
OTHER SUBSTANCES

Simultaneous: aprindine

REFERENCE

Misztal,G.; Przyborowski,L. Determination of aprindine in human plasma using reversed phase HPLC, *Pharmazie*, **1995**, *50*, 187–188.

Amiprilose



Molecular formula: $C_{14}H_{27}NO_6$
Molecular weight: 305.37
CAS Registry No.: 56824-20-5, 60414-06-4 (HCl)
Merck Index: 506

SAMPLE

Matrix: blood

Sample preparation: 250 μL Plasma + 100 μL 120 μg/mL IS in water, vortex for 1 min, add 200 μL 100 mM NaOH, add 6 mL dichloromethane, rotate for 15 min. Remove the lower organic layer and evaporate it to dryness under a stream of nitrogen, add 75 μL 26.67 mg/mL 2-chloro-1-methylpyridinium iodide in MeCN, add 200 μL 2.5 mg/mL 1,8-naphthalic dicarboxylic acid in MeCN containing 6 μL/mL triethylamine, heat at 65° overnight, cool, inject a 20 μL aliquot. (Prepare 1,8-naphthalic dicarboxylic acid by hydrolyzing 1,8-naphthalic anhydride with 5% NaOH (cf. Org. Syn. 1973, Coll. Vol. V, 813).)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultrasphere ODS (Altex)
Mobile phase: MeOH:1 M ammonium acetate:N,N-dimethyloctylamine:water 65:2.5:0.03:32.5
Flow rate: 1
Injection volume: 20
Detector: F ex 280 em 340

CHROMATOGRAM

Retention time: 21 (main peak, a minor peak due to the other conformer is also seen)
Internal standard: 1,2-O-isopropylidene-3-O-[3'-(N,N-diisopropylamino)ethyl]-α-D-glucofuranose (28)
Limit of quantitation: 185 ng/mL

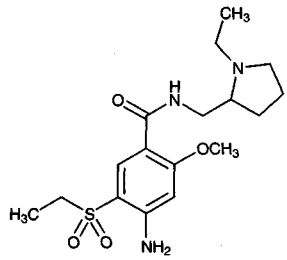
KEY WORDS

derivatization; plasma; pharmacokinetics

REFERENCE

Wu,S.T.; Benet,L.Z.; Lin,E.T. Determination of amprilose in human plasma by high-performance liquid chromatography with fluorimetric detection, *J.Chromatogr.B*, **1997**, *692*, 149–156.

Amisulpride



Molecular formula: $C_{17}H_{27}N_3O_4S$
Molecular weight: 369.49
CAS Registry No.: 71675-85-9
Merck Index: 508

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 20-65 μL 10 μg/mL tiapride + 200 μL 1 M NaOH + 10 mL chloroform, shake for 30 min, centrifuge at 2000 g at -10° for 15 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 25°, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm RP-18 (Altex, ODS II, Beckman)
Mobile phase: MeOH:water:diethylamine 532:468:0.8
Flow rate: 1
Injection volume: 50
Detector: UV 226

CHROMATOGRAM

Retention time: 10.3
Internal standard: tiapride (6.4)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Extracted: sultopride
Simultaneous: sulpride, nitrazepam, flunitrazepam
Noninterfering: metoclopramide, oxazepam

KEY WORDS

plasma

REFERENCE

Bohbot,M.; Doare,L.; Diquet,B. Determination of a new benzamide, amisulpride, in human plasma by reversed-phase ion-pair high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *416*, 414–419.

SAMPLE

Matrix: blood
Sample preparation: Add IS, extract with chloroform, back extract into an acidic medium, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Hypersil C18
Mobile phase: MeCN:MeOH:buffer 200:90:100 (Buffer was 1% triethylamine adjusted to pH 3 with phosphoric acid.)
Flow rate: 1.3
Detector: F ex 280 em 370

CHROMATOGRAM

Retention time: 4.4

Internal standard: present but not specified (7.8)
Limit of detection: 0.25 ng/mL
Limit of quantitation: 0.5 ng/mL

KEY WORDS
plasma

REFERENCE
Moulin,A.; Truffer,D.; Rauch-Desanti,C.; Istin,M.; Grognet,J.-M.; Dufour,A. Comparison of HPLC and RIA methods applied to the quantification of amisulpride in human plasma, *Eur.J.Drug Metab.Pharmacokinet.*, **1991**, *Spec No.3*, 507–512.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 225

CHROMATOGRAM
Retention time: 3.48
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifox-

amine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood

Sample preparation: Condition a 100 mg Isolute MF C18 (International Sorbent Technology) SPE cartridge with 2 mL MeCN, 2 mL water, and 500 μL buffer. 1 mL Plasma + 20 μL 10 μg/mL tiapride in MeOH + 1 mL buffer, vortex, add to the SPE cartridge, wash with 1 mL buffer, wash with 1 mL MeCN:water 30:70, wash with 80 μL MeOH, air dry for 30 s, elute with 500 μL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 250 μL mobile phase, inject a 100 μL aliquot. (Prepare buffer by dissolving 6.18 g boric acid and 7.46 g KCl in 1 L water. Mix 500 mL of this solution with 185 mL 100 mM NaOH, pH 9.)

HPLC VARIABLES

Guard column: 20 × 4.6 40 μm Pelliguard Si
Column: 250 × 4.6 Chiralpak AS amylose carbamate (J.T. Baker)
Mobile phase: n-Hexane:EtOH:diethylamine 67:33:0.2
Column temperature: 25
Flow rate: 0.5
Injection volume: 100
Detector: UV 280

CHROMATOGRAM

Retention time: 12 (S-(-)), 13 (R-(+))
Internal standard: tiapride (16)
Limit of quantitation: 2.5 ng/mL

KEY WORDS

mobile phase reservoir at 28°; plasma; chiral; SPE; pharmacokinetics

REFERENCE

Ascalone,V.; Ripamonti,; Malavasi,B. Stereospecific determination of amisulpride, a new benzamide derivative, in human plasma and urine by automated solid-phase extraction and liquid chromatography on a chiral column. application to pharmacokinetics, *J.Chromatogr.B*, **1996**, *676*, 95–105.

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. Condition a 3 mL C8 SPE cartridge (Analytichem) with 2.7 mL MeOH and 2.7 mL buffer A, do not allow to dry. 1 mL Plasma + 100 μL in 10 mM

HCl + 1 mL buffer A, mix, add to the SPE cartridge, rinse tube with 1 mL buffer A and add to the SPE cartridge, wash with 2.7 mL water, wash with 2 mL buffer B, dry for 1 min, wash with 200 μL acetone, dry for 30 s, elute with 1 mL buffer C. Add 50 μL buffer D to the eluate, evaporate to dryness under a stream of air, reconstitute in 200 μL mobile phase, sonicate for 1 min, inject an aliquot. Urine. Connect a Baker quaternary amine-silicane-bonded ion-exchange SPE cartridge on top of a Baker carboxylic-acid bonded silica gel SPE cartridge and condition with 1 volume buffer D, 1 volume water, 1 volume MeOH, and 1 volume water. 1 mL Urine + 1 mL water + 100 μL alpiropride in 10 mM HCl, mix, add to the SPE cartridges, rinse tube with 2 mL water and add rinse to the SPE cartridges, wash with 1 mL water, remove the top column, wash with one volume water, wash with two volumes MeOH, dry for 1 min, elute with 1 mL buffer D. Evaporate the eluate to dryness under a stream of air at 45°, reconstitute the residue in 200 μL mobile phase, sonicate for 1 min, inject an aliquot. (Buffer A was 10 mL triethylamine in 1 L water, pH adjusted to 7.00 with acetic acid. Buffer B was MeOH:water 20:80. Buffer C was 10 mL triethylamine and 7 mL acetic acid in 1 L MeOH. Buffer D was 2.10 mL concentrated HCl in 250 mL MeOH.

HPLC VARIABLES

Guard column: 10 mm long reversed-phase pellicular (Chrompack)
Column: 250 × 4.6 10 μm LiChrosorb RP-8
Mobile phase: MeCN:MeOH:buffer 16:8:76 (Buffer was 10 mL triethylamine in 760 mL water adjusted to pH 6.8 with acetic acid.)
Flow rate: 2
Injection volume: 175
Detector: UV 230

CHROMATOGRAM

Retention time: 4.9
Internal standard: alpiropride (6.0), amisulpride (4.9)

OTHER SUBSTANCES

Extracted: alizapride, metoclopramide
Simultaneous: acenocoumarol, acetaminophen, aspirin, caffeine, carbamazepine, clonazepam, codeine, isosorbide-5-mononitrate, nitrazepam, nitrofurantoin, theophylline
Noninterfering: amitriptyline, cisplatin, furosemide, indomethacin, isosorbide dinitrate, orphenadrine, propranolol

KEY WORDS

plasma; SPE; amisulpride is IS

REFERENCE

de Jong,A.P.; Wittebrood,A.J.; du Châtinier,W.M.; Bron,J. Liquid chromatographic analysis of alizapride and metoclopramide in human plasma and urine using solid-phase extraction, *J.Chromatogr.*, **1987**, *419*, 233–242.

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. Condition a 3 mL C8 Analytichem SPE cartridge with 1 volume (2.7 mL) MeOH and 1 volume buffer A, do not allow to dry. Mix 1 mL plasma + 100 μL 10 μg/mL alpiropride in 10 mM HCl + 1 mL buffer A, add to SPE cartridge, rinse the sample container with 1 mL buffer A and add the rinse to the SPE cartridge, wash with 1 volume water, wash with 2 mL buffer B, dry the column for 1 min, wash with 200 μL acetone, dry for 30 s, elute with 1 mL buffer C, add 50 μL buffer D, evaporate to dryness under a stream of air, reconstitute in 200 μL mobile phase, sonicate for 1 min, inject an aliquot. Urine. Connect a Baker 3 mL ion exchange quaternary aminesilicane-bonded silica gel SPE cartridge on top of a 3 mL Baker carboxylic acid-bonded silica gel SPE cartridge, condition with 1 volume (2.7 mL) buffer D, 1 volume of water, 1 volume of MeOH, and 1 volume of water. Mix 1 mL urine + 100 μL 10 μg/mL alpiropride in 10

mM HCl + 1 mL water, add to SPE cartridges, rinse the sample container with 2 mL water and add the rinse to the SPE cartridges, wash with 1 mL water, remove the top column, wash the bottom column with 1 volume of water and 2 volumes of MeOH, dry the column for 1 min, elute with 1 mL buffer D, evaporate the eluate to dryness under a stream of air at 45°, reconstitute in 200 μL mobile phase, sonicate for 1 min, inject an aliquot. (Buffer A was 10 mL triethylamine in 1 L water, pH adjusted to 7.00 with acetic acid. Buffer B was MeOH:water 20:80. Buffer C was 10 mL triethylamine + 7 mL acetic acid in 1 L MeOH. Buffer D was 2.10 mL concentrated HCl in 250 mL MeOH (100 mM).)

HPLC VARIABLES
Guard column: 10 cm long Chrompack reverse-phase pellicular material
Column: 250 × 4.6 10 μm LiChrosorb RP-8
Mobile phase: MeCN:MeOH:buffer 160:80:760 (Buffer was 10 mL triethylamine + 760 mL water adjusted to pH 6.8 with acetic acid (about 4.2 mL).)
Flow rate: 2
Injection volume: 175
Detector: UV 230

CHROMATOGRAM
Retention time: 4.9
Internal standard: alpiropride (6.0)

OTHER SUBSTANCES
Simultaneous: metoclopramide, alizapride, aspirin, theophylline, acetaminophen, caffeine, isosorbide-5-mononitrate, acenocoumarol, carbamazepine, nitrazepam, clonazepam
Noninterfering: indomethacin, orphenadrine, furosemide, cisplatin, amitriptyline, isosorbide dinitrate, propranolol
Interfering: codeine, nitrofurantoin

KEY WORDS
plasma; SPE; amisulpride is IS

REFERENCE
de Jong,A.P.; Wittebrood,A.J.; du Châtinier,W.M.; Bron,J. Liquid chromatographic analysis of alizapride and metoclopramide in human plasma and urine using solid-phase extraction, *J.Chromatogr.*, **1987**, *419*, 233–242.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a 100 mg Isolute MF C18 (International Sorbent Technology) SPE cartridge with 2 mL MeCN, 2 mL water, and 500 μL buffer. Mix 1 mL urine, 40 μg metoclopramide, and 25 mL water. Mix 1 mL plasma with 20 μL 40 μg/mL metoclopramide in MeOH. Mix 1 mL diluted urine or plasma with IS with 1 mL buffer, vortex, add to the SPE cartridge, wash with 1 mL buffer, wash with 1 mL MeCN:water 30:70, wash with 80 μL MeOH, air dry for 30 s, elute with 500 μL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 250 μL mobile phase, inject a 100 μL aliquot. (Prepare buffer by dissolving 6.18 g boric acid and 7.46 g KCl in 1 L water. Mix 500 mL of this solution with 185 mL 100 mM NaOH, pH 9.)

HPLC VARIABLES
Guard column: 20 × 4.6 40 μm Pelliguard Si
Column: 250 × 4.6 Chiralpak AS amylose carbamate (J.T. Baker)
Mobile phase: n-Heptane:EtOH:diethylamine 70:29.8:0.2
Column temperature: 28
Flow rate: 0.5
Injection volume: 100
Detector: F ex 280 em 370

CHROMATOGRAM

Retention time: 14.8 (S-(-)), 17.0 (R-(+))
Internal standard: metoclopramide (11.5)
Limit of quantitation: 2.5 ng/mL (plasma), 50 ng/mL (urine)

KEY WORDS

mobile phase reservoir at 34°; plasma; chiral; SPE; pharmacokinetics

REFERENCE

Ascalone,V.; Ripamonti,; Malavasi,B. Stereospecific determination of amisulpride, a new benzamide derivative, in human plasma and urine by automated solid-phase extraction and liquid chromatography on a chiral column. application to pharmacokinetics, *J.Chromatogr.B*, **1996**, *676*, 95–105.

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. Condition a 100 mg C18 (International Sorbent Technology) SPE cartridge with 2 mL MeCN, 2 mL water, and 500 μL buffer. 1 mL Plasma + 20 μL 5 μg/mL IS in MeOH + 1 mL buffer, vortex, add to the SPE cartridge, wash with 1 mL buffer, wash with 1 mL MeCN:water 30:70, air dry for 30 s, elute with 500 μL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 40-45°, reconstitute the residue in 250 μL 25 mM pH 3 KH_2PO_4, inject a 150 μL aliquot. Alternatively, mix 1 g plasma, 20 μL 2.5 μg/mL IS in MeOH, 1 mL water, and 200 μL 1 M NaOH, add 7 mL diethyl ether:chloroform 95:5, shake in a tumble extractor at 40 rpm for 10 min, centrifuge at 500 g for 8 min, freeze at -20°. Remove the upper organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 250 μL MeCN:25 mM pH 3 phosphate buffer 10:90, add 1 mL n-hexane shake in a tumble extractor at 20 rpm for 10 min, discard the hexane layer. Evaporate residual solvent under a stream of nitrogen at 40°, inject a 150 μL aliquot of the residual aqueous layer. Urine. Mix 1 mL urine and 20 μL 500 μg/mL IS in MeOH, dilute 100 fold with water. Mix 2 mL diluted urine and 200 μL 1 M NaOH, add 7 mL diethyl ether:chloroform 95:5, shake in a tumble extractor at 40 rpm for 10 min, centrifuge at 500 g for 8 min, freeze at -20°. Remove the upper organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 250 μL MeCN:25 mM pH 3 phosphate buffer 10:90, add 1 mL n-hexane shake in a tumble extractor at 20 rpm for 10 min, discard the hexane layer. Evaporate residual solvent under a stream of nitrogen at 40°, inject a 50 μL aliquot of the residual aqueous layer. (Prepare buffer by dissolving 6.18 g boric acid and 7.46 g KCl in 1 L water. Mix 500 mL of this solution with 185 mL 100 mM NaOH, pH 9.)

HPLC VARIABLES

Guard column: 20 × 4.6 40 μm Pelliguard LC8 (Supelco)
Column: 150 × 4.6 5 μm Hypersil C18 BDS
Mobile phase: MeCN:buffer 15:85 (Prepare buffer by mixing 25 mL 1 M KH_2PO_4, 950 mL water, and 1 mL triethylamine, adjust pH to 3 with phosphoric acid, make up to 1 L with water.)
Flow rate: 1
Injection volume: 50-150
Detector: F ex 280 em 370

CHROMATOGRAM

Retention time: 4
Internal standard: L-(-)-4-amino-N-[(1-ethylpyrrolidin-2-yl)methyl]-5-cyclopropylmethylsulfonyl-2-methoxybenzamide (Synthélabo Recherche) (7)
Limit of quantitation: 0.5 ng/mL, 100 ng/mL

KEY WORDS

plasma; SPE; pharmacokinetics

REFERENCE

Malavasi,B.; Locatelli,M.; Ripamonti,M.; Ascalone,V. Determination of amisulpride, a new benzamide derivative, in human plasma and urine by liquid-liquid extraction or solid-phase extraction in combination with high-performance liquid chromatography and fluorescence detection. Application to pharmacokinetics, *J.Chromatogr.B*, **1996**, *676*, 107–113.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 225.2

CHROMATOGRAM

Retention time: 8.923

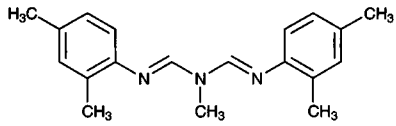
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Amitraz



Molecular formula: $C_{19}H_{23}N_3$
Molecular weight: 293.41
CAS Registry No.: 33089-61-1
Merck Index: 510
Lednicer No.: 4 36

SAMPLE

Matrix: formulations
Sample preparation: Dilute formulation 100-fold with MeOH, centrifuge at 1250 g for 10 min, inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 30 × 4.6 3 μm P-E 3 × 3 C18 (Perkin-Elmer)
Mobile phase: MeCN:water 85:15
Flow rate: 2
Injection volume: 10
Detector: UV 313

CHROMATOGRAM

Retention time: 0.73

OTHER SUBSTANCES

Also analyzed: chlorpyrifos (UV 313), coumaphos (UV 313), crotoxyphos (UV 229), permethrin (UV 229), phosmet (UV 229)

KEY WORDS

LOD 300 pg

REFERENCE

Rice,L.G. Rapid separation of pesticides by high-performance liquid chromatography with 3-μm columns, *J.Chromatogr.*, **1984**, *317*, 523–526.